In Vitro Model for Intestinal Uptake of Benzo[a]pyrene

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ABSTRACT

The human colon adenocarcinoma cell line, Caco-2, was used to study intestinal uptake of benzo(a)pyrene (BaP). BaP permeation was measured across Caco-2 monolayers grown on permeable supports that separate two chambers representing the intestinal lumen and the bloodstream. At high BaP concentration (10 μM) BaP permeation of the cell layer occurred at a linear rate. At lower and physiologically more relevant BaP concentrations (0.2 and 1 μM) permeation showed a more complex pattern with initial linear rate, then accelerated and finally reduced permeation. From the earliest sampling on (0.5 h) BaP permeation of the cell layer was accompanied by extensive metabolism to water soluble conjugates not extracted by organics. With 0.2 μM BaP about half the ^{3}H-labeled material appearing in the basolateral chamber consisted of BaP conjugates, and of the ^{3}H-material extracted with organic solvents about half consisted of BaP metabolites. In addition we found that Caco-2 cells preferentially released metabolites into the apical chamber representing the intestinal lumen. We conclude that the intestinal epithelium is an important barrier that limits systemic availability of ingested BaP by presystemic detoxification and outwardly directed transport. We also conclude that the Caco-2 cell system is a useful in vitro model to predict systemic availability of xenobiotics in general.

Abbreviations: BaP, benzo[a]pyrene; PAH, polycyclic aromatic hydrocarbon; PEG, polyethylene glycol; Pgp, P-glycoprotein; PhPI, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine.

INTRODUCTION

Several polycyclic aromatic hydrocarbons (PAHs)[1] are known or suspected human carcinogens, including benzo[a]pyrene (BaP), the most widely studied of all PAHs (*1*). PAHs require metabolic activation to exert their oncogenic effect. Two large families of enzymes perform the metabolism. Phase I enzymes represented by cytochrome P450s activate compounds to reactive, electrophilic intermediates (*2*). For BaP this pathway generates the ultimate carcinogen, 7,8-diol-9,10-epoxide which binds to DNA, causing bulky adducts that can lead to mutations. Phase II enzymes conjugate the intermediate with hydrophilic compounds, and the conjugate can be excreted, therefor this step is considered detoxification. Major human exposure routes to PAHs are inhalation of polluted air and tobacco smoke and ingestion of PAH-containing food. The total BaP dose has been assessed in a population exposure study in nonsmokers, and even though large interindividual and seasonal changes were observed, overall the BaP dose taken in through the diet was 5-50 times greater than the dose through inhalation (*3*), whereas among smokers inhalation and ingestion each account for half the BaP intake.

Ingested PAHs are taken up from the intestinal lumen into the vascular circulation across the gastrointestinal epithelial lining, which regulates the rate of absorption and bioavailability of a compound. Most drugs traverse the intestinal epithelium by passive diffusion, either through cells (transcellular) or between cells (paracellular). PAHs, as all lipophilic compounds, permeate mainly by the transcellular route. An understanding of intestinal transport of environmental contaminants is important to assess their actual bioavailability. For some compounds the systemic availability might only be a fraction of the ingested dose.

Various animal model systems are used to study the intestinal transport. An alternative to these time-consuming, expensive animal studies is the use of monolayers of human intestinal-type cells grown on microporous filters (*4, 5*). The membrane on which these cells are grown separates two chambers representing the intestinal lumen (upper chamber) and the bloodstream (lower chamber). Permeation of a compound across the intestinal cell layer can be measured by adding a compound to the upper chamber and monitoring its appearance in the bottom chamber. The cells of choice used in this in vitro model are the Caco-2 cells. This cell line was derived from a human colorectal carcinoma and differs from other cell lines of the same origin in that under conventional cell culture conditions it spontaneously differentiates into monolayers of polarized columnar cells analogues to the small intestine (*5*). Caco-2 monolayers exhibit well-developed microvilli, have tight intercellular junctions, active transport carriers and express high levels of several brush border hydrolases on the apical surface (*6*). They also express various cytochrome P450 isoforms, such as CYP1A1 and CYP3A4, and phase II enzymes, such as uridine diphosphoglucuronosyltransferase, sulfotransferases and glutathione-S-transferases (*6*). Therefore, they exhibit many properties of the enterocytes of the small intestine. Caco-2 cell monolayers have been shown to represent a reproducible in vitro model of the intestinal epithelium and to give useful prediction on the absorption of compounds. A good relationship between permeability in Caco-2 monolayers and human absorption has been reported, and the transport of small organic drugs roughly correlates with human bioavailability (*7, 8*). Therefore the Caco-2 system is frequently used to predict intestinal uptake of pharmaceutical drugs.

We have used the Caco-2 cell system to simulate intestinal uptake of BaP as model compound for PAHs. We find that BaP permeation of the cell layer is accompanied by extensive metabolism leading to partial detoxification before being released into the lower chamber that represents the bloodstream. We conclude that the systemic availability of ingested xenobiotics dependents not only on the physico-chemical forces that determine the bioavailability, but also on the barrier function of the intestinal layer of cells that limits the uptake of potentially harmful xenobiotics.

MATERIALS and METHODS

Cells

Caco-2 cells (passage 19) were obtained from the American Tissue Culture Collection, Rockville, MD. Cells were maintained in Eagle's Minimum Essential medium with non-essential amino acids and Earle's salts supplemented with 10% fetal calf serum, 1 mM L-glutamine, 10 mM Hepes and antibiotics. Cells were subcultured 1 : 10 every 7 days. Cells between passage 26 to 40 were used in all experiments.
For transport studies Caco-2 cells were seeded at 3 x 10^5 cells into polycarbon (or polyester) membrane cell culture chambers (Transwell, 0.4-µm-pore, 4.7 cm^2, Corning Costar, Cambridge,MA) containing 2.5 ml medium in the lower compartment, 1.5 ml in the upper compartment . The medium was changed every other day. The formation of functional epithelial layers was monitored visually when polyester transwells were used.
The integrity of the monolayers was determined by following the transepithelial transport of a non-metabolizable macromolecular marker, polyethylene glycol (PEG, MW 4000). ^{14}C-PEG_{4000} (spec. activity 15.3 mCi/g, Amersham, Arlington Heights, IL) was added to the apical chamber and 20 µl aliquots taken from the basolateral chamber every 30 min. ^{14}C-activity was determined in a liquid scintillation counter.

Transport experiments

Confluent monolayers seeded 20 to 30 days before were used for transport experiments. ^3H-BaP, generally labeled with tritium (69 Ci/mmol, Amersham, Arlington Heights, IL) was dissolved in DMSO and diluted in medium to give a 0.2 µM solution. For higher concentrations unlabeled BaP was added to give the final concentration. The final DMSO concentration in the apical chamber was 0.1 %. Before the experiment the cell layers were rinsed with HBSS and received fresh medium. Then the ^3H-BaP solution was added very carefully to the apical chamber. The plates were agitated on a Rotamix at 40 rpm. The medium in the basolateral chamber was replaced at intervals either by removing the medium through the sample port or by lifting the insert containing the cell layer and apical solution to a fresh compartment. Appearance of ^3H-activity was analyzed by liquid scintillation counting.

From the ^3H-activity measurements the apparent permeability coefficient (P_{app}) was calculated as described in (*8*) using the equation

$$P_{app} = dQ/dt \times 1/A \times C_o$$

where dQ /dt is the flux of the compound across the monolayer per second (flux is defined as the steady-state rate of appearance of apically applied compound on the

basolateral site of the cell layer), A is the surface area of the cell layer and C_O is the initial concentration of the compound.

Analysis of ^{3}H-BaP metabolites

The medium was extracted with 2 volumes cold ethyl acetate/acetone (2:1 v/v) containing α-tocopherol (0.1 mM) as an antioxidant. The aqueous and organic phase were separated and aliquots taken to determine ^{3}H-activity by liquid scintillation counting. To determine the amount of ^{3}H-BaP and metabolites in the cell layer, cells were washed three times with SSC, scraped off the membrane, suspended in SSC and lysed by adding 10% SDS to make the solution 1%. This solution was then extracted with 2 volumes ethyl acetate/acetone.

The organic phase was further analyzed by reversed-phase HPLC as described in (*9*). Aliquots of the organic extracts were concentrated under a stream of nitrogen. HPLC analysis was performed on a Beckman apparatus equipped with a Waters Radial-Pak C-18 column eluted by a methanol /water gradient at a flow rate of 1 ml/min for 60 min. Fractions were collected at 1 min intervals and the elution profile determined by liquid scintillation counting. Peaks were identified by comparison to the elution of known BaP metabolite standards.

The ^{3}H-material remaining in the water phase after extraction with ethyl acetate/acetone and consisting of conjugated PAH metabolites was in some instances analyzed further as described in (*10)*. Remaining organic solvent was removed by gently blowing a stream of N_2 over the solution, and the pH of the solution was adjusted to 5. The solution was then treated with 500 units per ml β-glucuronidase (from Helix pomatia; Sigma, St.Louis, MO) and 20 units per ml sulfatase (from Helix pomatia; Sigma, St.Louis, MO) for 20 hr at 37^{o}C. After incubation samples were extracted 2 times with 2 volumes ethyl acetate/acetone as described above and the ^{3}H-material partitioning into the organic phase after enzyme treatment was determined.

RESULTS

1. Characterization of cell layers

Initially Caco-2 cells were grown in transwells with either polycarbonate or polyester membranes. The advantage of the polyester is that it is transparent, and one can monitor the cell growth under the microscope. As described by others (*4*) cells appeared large with irregular shapes in the first days after seeding. Once cells reached confluence their shape appeared more regular. As the culture got older domes formed between areas of monolayer.

When performing the BaP transport experiments as described below we found that BaP permeation of Caco-2 layers grown on polyester is reduced by about a factor of 2 compared to polycarbonate membranes. Therefore we used only polycarbonate membranes for all experiments described here. We did not investigate the reason for the different permeability of Caco-2 monolayers grown on polyester versus poycarbonate membranes any further.

To establish that Caco-2 cells had formed a continuous monolayer and to determine the transepithelial permeability, ^{14}C-PEG was used as leakage marker. ^{14}C-PEG was added to the apical compartment and appearance of ^{14}C in the basolateral compartment was determined. Periodically these measurements were done at the end of a BaP

transport experiment to ensure that the integrity of the cell layer was not affected by the BaP. We
consistently found that permeation of the cell layer was less than 0.5 % of added ^{14}C-PEG per hour.

2. BaP permeation across Caco-2 layer as function of dose and time

Monolayers of Caco-2 cells in polycarbonate cell culture chambers were exposed to different concentrations of BaP by adding 1.25 ml of 0.2, 1, or 10 μM ^{3}H BaP solutions to the apical chamber of a transwells (the volume in the apical chamber was reduced from the 1.5 ml used during culturing to 1.25 ml to reduce any chance of accidental spilling into the basolateral chamber). The medium was changed every 30 min for 8 hours and the appearance of ^{3}H -activity in the basolateral chamber was measured. The results presented in Figure 1 a-c represent two independent experiments and each value is the mean of two transwells. BaP permeation of the Caco-2 cell layer occurred at a fairly constant rate after addition of 10 μM BaP. However, at lower BaP concentrations permeation of the cell layer showed a more complex pattern. After an initial constant rate of BaP permeation the rate increased at 2.5 h (0.2 μM BaP) or 3.5 h (1 μM BaP) after BaP addition, then permeation decreased to a fraction of the original level. This decreased permeation occurred even though the apical chamber still contained about a 50-fold higher concentration of BaP. The peak permeation depended not only on the BaP concentration, but to some extent on the age of the cell layer. In one experiment the cell layers were two weeks older than those in Figure 1. Even though the same general pattern of permeation

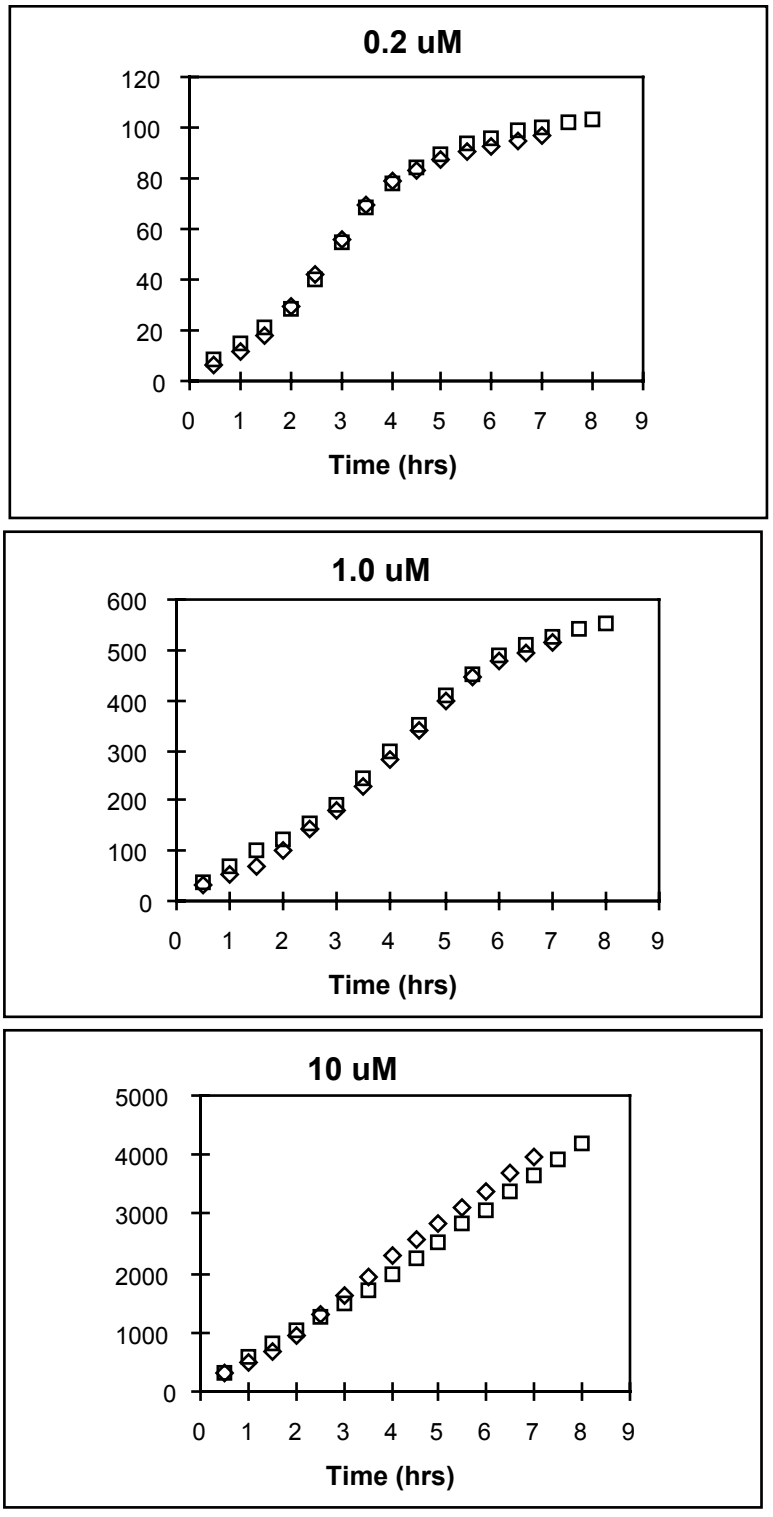

Figure 1: ^{3}H-BaP permeation of Caco-2 cell layers after BaP addition to apical chamber (top panel - 0.2 μM BaP, middle panel - 1 μM BaP, bottom panel - 10 μM BaP). Values from two independent experiments, each the mean of two transwells, are presented. In the first (◇) medium was collected and replaced through the sample port every 30 min

for 7 hrs. In the second (□) transwells were lifted every 30 min into fresh wells for 8 hrs.
was observed, the peak permeation was one hour later (data not shown). After 8 h permeation of the cell layer about 60% of the total BaP added still remained in the apical chamber or the cell layer. We have observed consistently that ^{3}H-activity in the basolateral chamber is increased at the very first sampling compared to the following ones. This could possibly be due to a disturbance of the cell layer when adding the solution.
From the initial permeation rate we calculated the apparent permeability coefficient (P_{app}) as described in the Material and Methods section. The calculated P_{app} value slightly decreased with increasing BaP concentrations: for 0.2 µM 4.3 x 10^{-6} cm/s, for 1 µM 3.8 x 10^{-6} cm/s, and for 10 µM 2.9 x 10^{-6} cm/s. These values are close to the one found by others (P_{app} =1.8 x 10^{-6} cm/s, Hsu, personal communication).

3. BaP metabolism during permeation of Caco-2 layer

To determine if the ^{3}H-radioactivity appearing in the basolateral chamber was intact BaP or if some metabolic transformation had occurred, the medium collected in the above experiment was extracted with ethyl acetate/acetone and the distribution of ^{3}H-material between the aqueous and organic phase was measured. It is well established that the more hydrophilic conjugated BaP metabolites remain in the aqueous phase, whereas BaP partitions into the organic phase (*10*). As seen in Figure 2, a considerable fraction of ^{3}H-labeled material appearing in the basolateral chamber consists of water soluble BaP-conjugates, especially at the lowest BaP concentration.

The fraction of water soluble material is reduced with increasing BaP concentrations and also shows a temporal variation. The highest fraction of water soluble material is always observed at the initial sampling, followed by a reduced level and a concentration-dependent recovery.

The ^{3}H-material in the organic phase could be either unmetabolized BaP or BaP metabolites, such as diol and tetrols. To test this further medium containing 0.2 µM BaP was added to the apical chamber of a transwell and after one hour the medium from the apical and basolateral chambers, as well as the cell layer were collected and extracted with ethyl acetate/acetone and the ^{3}H- material in the organic phase was analyzed by HPLC. The elution profiles (Figure 3) show that much of the ^{3}H-labeled material has been metabolically transformed. With the fresh BaP medium (before addition to the chamber)

97 % of the ^{3}H -activity was eluted in the BaP peak. About 90 % of total activity was eluted in the BaP peak in case of both the medium from the apical chamber and the extract of the cell layer, whereas less than 40 % of the ^{3}H-activity was eluted in the BaP peak in case of the medium from the basolateral chamber. Therefore after addition of 0.2 µM BaP to the apical side of the Caco-2 layer more than half of the ^{3}H-material recovered from the basolateral chamber consists of conjugated BaP metabolites, and of the remaining ^{3}H-activity that can be extracted with ethyl acetate/acetone roughly half has been metabolized. So a large proportion of the ^{3}H-activity in the basolateral chamber consists of metabolites and less than 25 % is unmetabolized BaP. Whereas the metabolites in the basolateral and apical medium consist mainly of tetrols, the cell layer contains several other metabolites.

4. Distribution of BaP and metabolites between compartments

The distribution of ^{3}H-material between apical, basolateral chamber and cell layer was determined 0.5 and 2h after addition of ^{3}H-BaP to the apical chamber. The medium in the basolateral chamber was collected every 30 min. At 0.5 and 2 h after addition of 1 ml 0.1, 0.5 and 5 µM ^{3}H-BaP to the apical chamber, one cell culture chamber each was analyzed. The total ^{3}H-radioactivity of the medium in the apical and basolateral chambers and in the cell layer were determined. In case of the 2 h time point the medium collected every 30 min was combined and analyzed. The results in Table 1 representing the mean of two experiments show that BaP is rapidly taken up into the Caco-2 cell layer, so that after only 30 min 35-45 % of the ^{3}H-label is in the cell fraction, but only 2 - 3 % in the basolateral

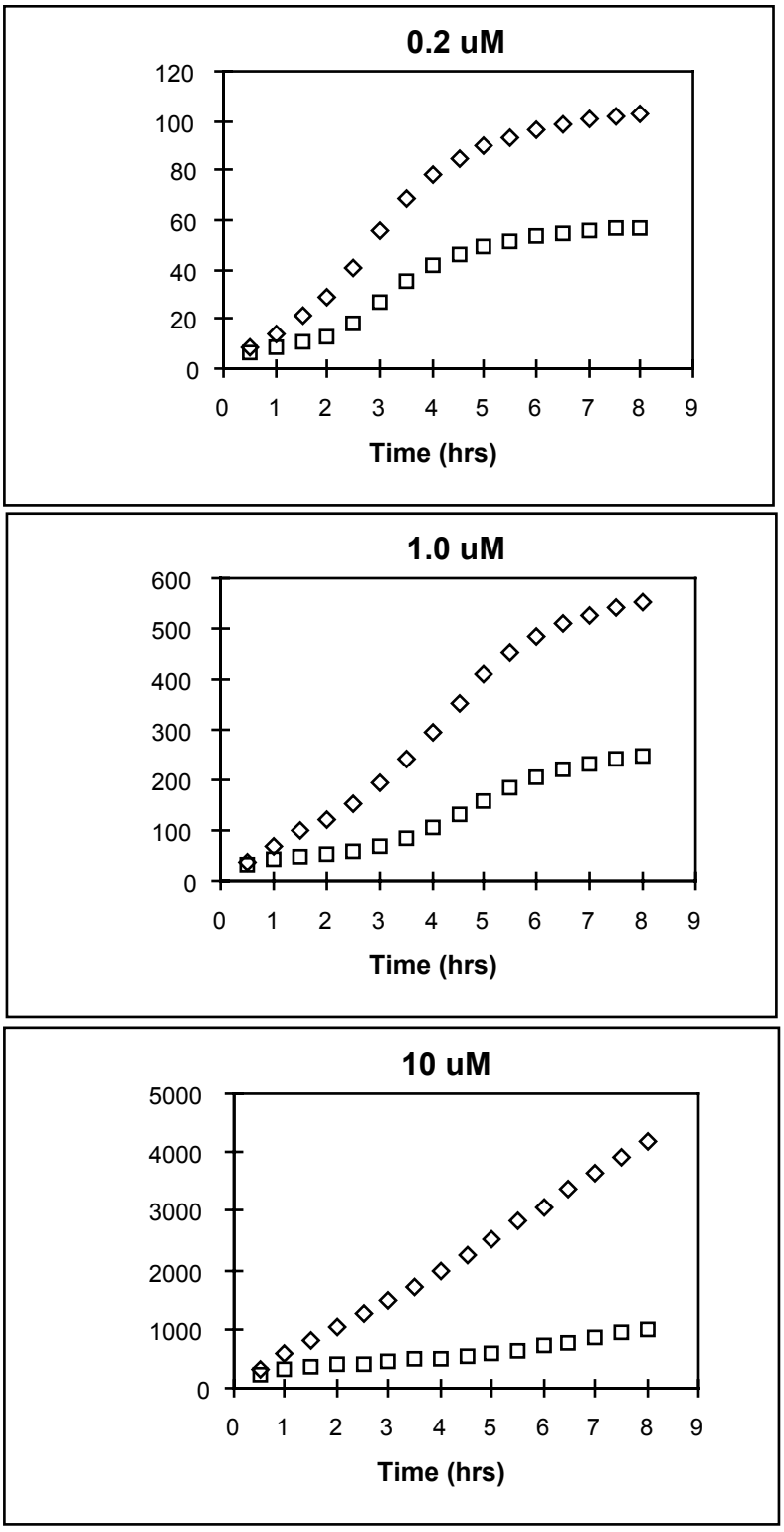

Figure 2: Water soluble BaP metabolites appearing in the basolateral chamber after addition of different concentrations of BaP to apical chamber (top panel - 0.2 μM BaP, middle panel - 1 μM BaP, bottom panel - 10 μM BaP). Total ^{3}H-activity recovered from

the basolateral chamber (◈◆) and ^{3}H -material remainining in the aqueous phase (□) after extraction with ethyl acetate/ acetone were determined.

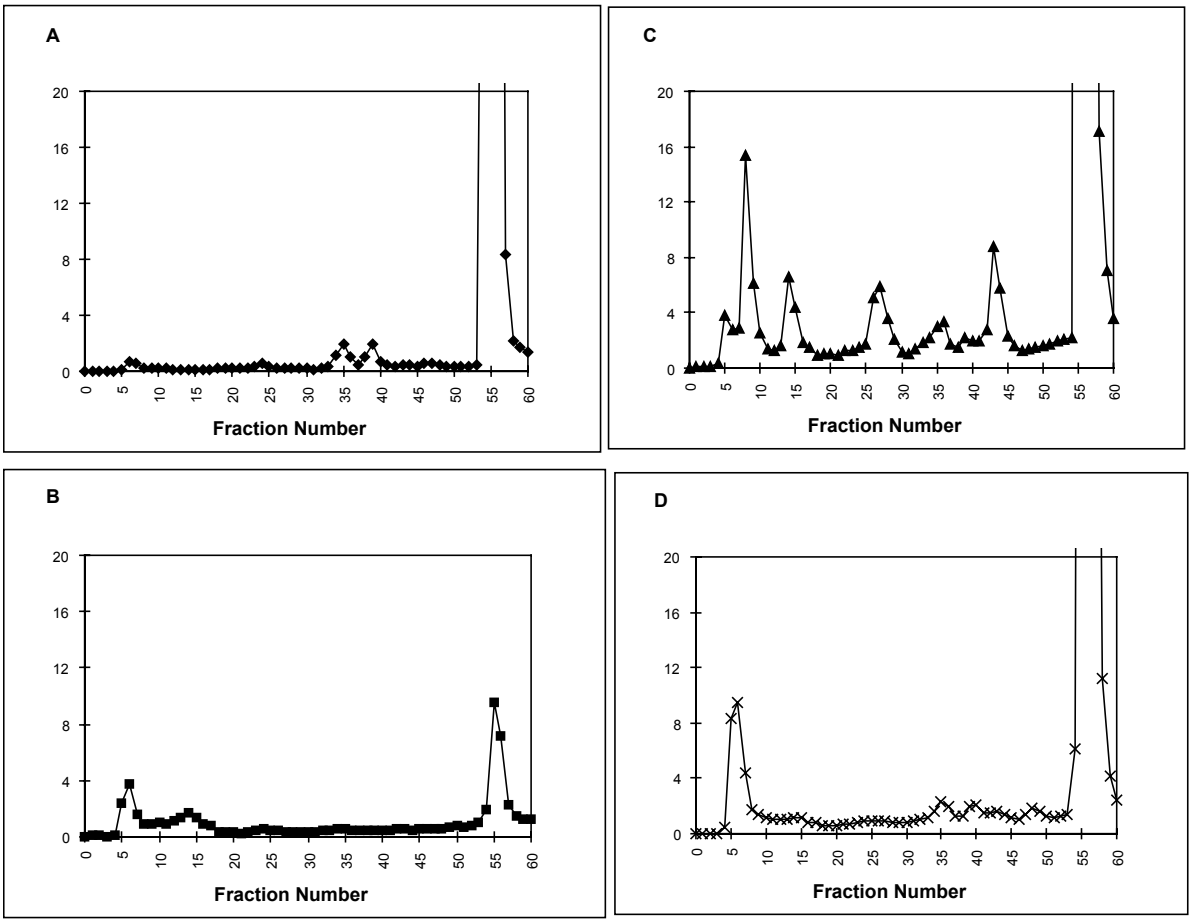

Figure 3: HPLC profile of organic extracts of (A) BaP medium before addition to transwell, (B) basolateral medium, (C) cell layer, and (D) apical medium 1 hour after addition of 0.2 μM ^{3}H -BaP to apical chamber**.** Peaks were identified by comparison to the elution of known BaP metabolite standards. Various tetrol and triol isomers are eluted in the first 18 fractions, followed by diols, then monol and finally BaP.

chamber. After 2 h about half of the ^{3}H-material is in the cell layer and 13 - 15 % in the basolateral chamber.

Each sample was then extracted with ethyl acetate/acetone and the distribution of ^{3}H-label between aqueous and organic phase was determined. Again half to a third of the ^{3}H -label in the basolateral chamber consisted of water-soluble material. This value decreased for the pooled 2 hr collection medium. Whereas the apical medium and cell layer contained very little water-soluble material at 0.5 h, the fraction of water-soluble material had increased considerably at 2 h. The absolute amount of water-soluble BaP metabolites recovered from the basolateral and apical chambers was calculated from the ^{3}H-label (Table 2). Clearly, the apical compartment contains more water-soluble BaP

metabolites than the basolateral indicating that Caco-2 cells preferentially release metabolites into the apical chamber. There are also differences in the composition of the ^{3}H-material in the aqueous phase. Whereas 33-39 % of ^{3}H-labeled material from the aqueous phase of the apical chamber was digested by β-glucuronidase and sulfatase treatment, only 16-24 % of ^{3}H -labeled material from the aqueous phase of the basolateral chamber was digested by this treatment. As the material resistant to β-glucuronidase and sulfatase digestion is considered to be glutathione conjugates of BaP metabolites (*10*), these data indicate that more glutathione conjugates are released into the basolateral chamber than into the apical chamber. Therefore Caco-2 cells show a vectorial transport in that metabolites are preferentially released into the apical chamber, and in that there is a qualitative difference in metabolite composition between apical and basolateral compartment.

Table 1: Distribution of ^{3}H label between compartments 0.5 and 2 hr after BaP addition expressed as % of total radioactivity, in parenthesis fraction of ^{3}H label remaining in aqueous phase after extraction with ethyl acetate /acetone. The values represent means of two experiments.

Time after BaP addition	Compartment	0.1 µM BaP	0.5 µM BaP	5.0 µM BaP
0.5 hr	apical	56.8 (0.09)	61.2 (0.07)	54.3 (0.07)
	cell layer	40.2(0.05)	36.2 (0.07)	43.7 (0.03)
	basolateral	3.0(0.46)	2.7 (0.39)	2.0 (0.3)
2.0 hr	apical	48.0 (0.2)	43.3 (0.11)	40.1 (0.09)
	cell layer	37.5 (0.14)	43.4 (0.07)	46.8 (0.04)
	basolateral	14.5 (0.34)	13.4 (0.23)	13.61 (0.12)

DISCUSSION

Human exposure to PAHs occurs to a large degree by ingestion (*11*). The amount of ingested BaP depends on diet and cooking method. One study estimated an adult daily intake between 1.5 - 570 ng BaP (*12*), but if charcoal-broiled food is a regular part of the

diet, BaP intake could be even higher (*13*). The liver is the major site for the metabolic activation and detoxification of ingested PAHs (*14*). After intravenous injection of BaP to rats or mice, maximum metabolic alteration occurs in the hepatobiliary system and the primary route of BaP elimination from the host is through the feces (*15*). However, after ingestion detoxification may also occur to some extent in the intestinal epithelium. The importance of the route of BaP administration for the carcinogenic response was shown by Weyand et al.(*16*) in mice: whereas a single ip dose of BaP induced lung tumors in all animals, ingestion of a diet containing BaP resulted only in a small, statistically not significant increase in lung tumors.

Table 2: Amount of conjugated BaP (pmole) released into apical and basolateral compartment. The numbers in parenthesis represent the fraction of ^{3}H-material digested by sulfatase and β-glucuronidase.

Time after BaP	Compartment	0.1 µM BaP	0.5 µM BaP	5.0 µM BaP
0.5 hr	**apical**	**4.6 (0.35)**	**22.5 (0.36)**	**211.6 (0.37)**
	basolateral	**1.2 (0.16)**	**5.1 (0.19)**	**39.6 (0.22)**
2.0 hr	**apical**	**8.8 (0.33)**	**24.3 (0.35)**	**187.5 (0.39)**
	basolateral	**3.7 (0.18)**	**10.7 (0.20)**	**37.6 (0.24)**

The intestinal cell layer is equipped with a number of enzyme systems involved in detoxification of xenobiotics, since one of the functions of the intestinal mucosa is to protect the body against toxic and carcinogenic compounds. In normal rat intestinal epithelium some members of the family of cytochrome P450 mono-oxygenases (CYP1A1, CYP1B1 and CYP3A), as well as glutathione *S*-transferases and uridine diphosphoglucuronosyltransferase are expressed (*17, 18*), and expression of various

enzymes including CYP1A1 is induced by BaP. CYP1A1 activity in small intestine might be higher in humans than in rats: when BaP content in feces of rats and human volunteers was compared after consumption of charcoal-broiled hamburgers, 11 % of BaP was excreted unchanged in rats whereas no BaP was detected in human feces (*13).* Recently a large interindividual variation of CYP1A1 expression in human small bowel has been reported (*19*). A 100-fold interindividual variation in BaP metabolism of cultured human colon has long been known (*10*).

We have used the Caco-2 system, a well-accepted model of human intestinal absorption, to investigate intestinal absorption of ingested BaP. We extended the measurement of BaP permeation of the Caco-2 cell layer over a 7-8 h interval. This is longer than the residence time of food in the intestine (about 4 h), but we tried to simulate a maximum exposure scenario defined by continuous presence of BaP in successive meals in order to include the contribution of inductive processes. Lipophilic compounds like BaP are transported across the intestinal mucosa by the transcellular route along the concentration gradient from the intestinal lumen into the bloodstream. When drug transport measurements are performed, usually a linear rate of permeation is observed (*5*). In the present study we determined permeation of BaP at three different concentrations. We found a linear permeation of BaP over the 8 h-interval at the highest concentration (10 μM), but for the 10- and 50 fold lower doses this was only true in the first 2-3 hours (Figure 1). The change in the rate of permeation over time was most pronounced at 0.2 μM BaP which probably represents a physiologically relevant dose, whereas at 10 μM BaP, the rate of permeation appeared constant. The data suggest that BaP at doses below 10 μM does not simply cross the cell layer by passive diffusion, but that active processes are involved that first accelerate, then reduce permeation. The later could be due to the multidrug resistant P-glycoprotein being induced.

Only a fraction of the ^{3}H-labeled material permeating the Caco-2 cell layer and appearing in the basolateral chamber is unmetabolized BaP, but much of the BaP seems to be metabolized by the cells before reaching the basolateral chamber. Since conjugation renders the BaP metabolites less reactive the conjugates are in most cases considered less toxic. The data in Figure 2 suggest that much of the BaP reaching the basolateral side has been detoxified to conjugated BaP metabolites. With 0.2 μM BaP about half the ^{3}H-labeled material consisted of water soluble BaP-conjugates. Analysis of the metabolites in the organic fraction showed that the Caco-2 cell layer contained tetrols and diols. If such metabolic activation occurs in the intestine, these intermediates can then be transported distally to regions where the diol could be detoxified or form the ultimate carcinogen, a diol epoxide. The second possibility is especially interesting because CYP1B1 which has a different tissue distribution than CYP1A1, has a higher activity for diols than for the parent compound (*18*). In case of the cooked-food mutagen 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhIP) no metabolic conversion was observed during transport across the Caco-2 cell layer (20), but this compound is activated primarily by CYP1A2, a liver-specific enzyme.

We found that the medium in the apical chamber contains an even higher amount of water soluble BaP metabolites than the basolateral chamber. This suggests that Caco-2 cells actively pump the BaP metabolites to the apical surface. This may be due to P-glycoprotein (Pgp), a membrane protein that mediates active transport of various lipophilic substrates. Pgp was first described in cancer cells, where it is one of the underlying causes of resistance to chemotherapy (*21*), but it also has a physiological

function. In normal human tissue Pgp is localized to the luminal surface of cells lining the small intestine, colon and proximal tubes of the kidney (*22*). This observation indicates that Pgp functions to limit the absorption of toxins. Caco-2 cells express Pgp and the expression has been localized to the apical brush-border (*23*). Since expression of Pgp seems to affect the susceptibility of MCF-7 cells to BaP, a role of Pgp has been proposed as efflux pump for BaP (*24*). ATP-dependent transport of BaP has been demonstrated in apical membrane vesicles from normal human intestinal cells (*25*). The transport of glutathione conjugates out of Caco-2 cells occurs by a carrier-mediated, ATP-dependent process and this secretion is highly asymmetric (*26*). Therefore it seems possible that the observed qualitative and quantitative differences in metabolites in apical and basolateral compartments are due to Pgp preventing BaP metabolites from permeating the cell layer. However a recent report (*27*) did not find evidence for a role of Pgp in BaP translocation in cell lines stably expressing Pgp. In the case of PhIP efflux was shown to be a true active process and inhibitors of Pgp and of the multidrug resistance-associated protein increased PhIP permeation of Caco-2 cells suggesting that PhIP is a substrate for these transporters (*20*). Further studies are needed to clarify if Pgp influences retention of BaP.

A major function of the intestinal epithelium is to absorb nutrients, electrolytes and water, while at the same time serving as a barrier to potentially harmful compounds ingested. We have found significant metabolic transformation and detoxification of PAHs by intestinal cells. This presystemic metabolism could significantly reduce the risk of ingested contaminants. If one can extrapolate from the Caco-2 monolayer model to the in vivo situation, much of the ingested BaP taken up by the intestinal mucosa is metabolically transformed to non-toxic metabolites before reaching the blood stream. In addition, metabolites are released preferentially into the intestinal lumen. Therefore the intestine could have a major protective role against the toxicity of PAHs and reduce the systemic availability of these compounds to a fraction of the ingested dose.

The Caco-2 monolayer system is a well-accepted model of human intestinal absorption and is used extensively to predict oral availability of pharmaceutical drugs. The system appears equally useful to evaluate oral availability of ingested xenobiotics. Of course the in vitro system has limitations. It cannot address complex issues such as influence of diet and lipid content on intestinal transport or differentiate between absorption of compounds into the lymphatic or vascular circulation as has been observed for PAH intestinal absoption (*28*). Still the Caco-2 system can provide useful estimates on rate of absorption, presystemic metabolism of compounds and on interaction of different environmental compounds without the need for time-consuming animal experiments.

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